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Antioxidant Protective Effect of *Malva sylvestris* on Vanadium Induced Testicular Oxidative Stress in Male Wistar Rats

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Abstract

Vanadium can affect the reproductive functions of male rats. Many medicinal plants especially Malva sylvestris, rich in phenolic compounds, possess antioxidant properties. Eighty rats were exposed to ammonium metavanadate (0.24 mmol/kg body weight in drinking water) for 90 days. Chronic vanadium intoxication induced a low body weight gain. Testis histological examination revealed atrophy of seminiferous tubules and defects of spermatogenesis. Spermatozoa disappeared. Lipid peroxidation level and superoxide dismutase, catalase and glutathione peroxidase were examined in testis. Chronic vanadium intoxication induced a low body weight gain. Testis histological examination revealed atrophy of seminiferous tubules and defects of spermatogenesis. Spermatozoa disappeared. Lipid per-oxidation was significantly increased but no significant change in antioxidant enzyme activities was shown. In vanadium-treated rats given *Malva sylvestris* decoction, no such pathological features were observed.

Our results revealed that *Malva sylvestris*, rich in phenolic compounds, possess a high anti-oxidative potential.

Keywords: Lipid peroxidation; Malva sylvestris; Oxidative stress; Rats; Testis; Vanadium

Introduction

Vanadium is a trace element present in the environment and widely distributed in the animal and plant kingdom [1]. The trace element compounds are disturbed in water, rocks, and soil as well as in fossil fuels [2,3]. The main source of exposure to vanadium for the general population is food in which a few tens of micrograms of daily dietary intake were estimated [1]. Even though relatively high, vanadium concentrations have been recorded. Drinking water contributes to some water supplies especially in ground-water from volcanic areas [4]. The use of dietary mineral supplements is another important source of vanadium exposure that can provide more than 10 mg vanadium/day [5]. Vanadium compounds have the capacity to regulate different physiological processes such as cell growth, differentiation and glucose and lipid metabolism [6]. Vanadium, at low concentrations, may also cause several adverse effects on mammals such as hemopoietic changes, nephrotoxicity, reproductive and developmental toxicity [7,8]. A variety of chemical compounds, including metals, have the potential to alter certain stages of the spermatogenesis process, leading to reproductive dysfunction [9,10] and fertility impairment [3,10]. Moreover, vanadium has been reported to induce low sperm counts, to decrease sperm motility, and to increase the frequency of sperm morphology abnormalities [9]. Vanadium compounds have been shown to induce Reactive Oxygen Species (ROS) [11] production and alteration of the

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anti-oxidative activity of enzymes such us Superoxide Dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx) [12].

The ancient medical systems have suggested that a number of plants and herbs have been used to reduce the toxicity of various pollutants especially vanadium and to protect oxidative stress. Vanadate salts were ingested with botanic antioxidants namely mallow decoction [12] or green tea [13]. Several studies confirm the interaction between flavonoids and other plant-derived polyphenolic compounds with antioxidant and free radical scavenging properties [14]. Flavonoids are a group of naturally occurring compounds widely distributed in of plant origin, providing flavor and color to fruits and vegetables [15]. These natural compounds are reported to have foods antioxidant, anti-inflammatory and anti-proliferative effects, which could cause possible involvement with the development of diseases [16]. As antioxidants, flavonoids can prevent oxidative injury [17] and contribute to the delicate balance between oxidative and antioxidant processes in tissues [18]. Flavonoid compounds are commonly found in most medicinal plants and exert a remarkable spectrum of biological activities which affect basic cell functions [19].

Malva sylvestris, a traditional medicinal plant, was used in traditional phototherapy and cosmetic treatments [20]. Fluid extract of *Malva sylvestris* flowers and leaves are used as a valuable remedy for cough and inflammatory diseases of mucous membranes [21]. The biological activity of this plant may be attributed to its richness in antioxidants [22]. Phytochemical studies on mallow have shown that its various parts contain flavonoids [23,24], terpenoids [25], phenol derivatives [23-25], polysaccharides [26], mucilages and coumarins [27], vitamins C and E and beta-carotene [23]. Besides, this plant also appears to have therapeutic properties [28,29]. It can have anti-diabetic [30], anti-inflammatory [31], anti-ulcerogenic [32], and anti-oxidant effects [33,34]. Indeed, *Malva sylvestris* leaf extract is known for its ability to scavenge Reactive Oxygen Species (ROS), to exert a neuroprotective effect by reducing lipid per-oxidation level in kidney and to enhance the efficiency of the endogenous antioxidant system.

Hence, the current study was designed to evaluate, in rats, the protective effects of *Malva sylvestris* on reproductive system damage induced by vanadium toxicity in the rat.

Materials and Methods

Plant material

The leaves of *Malva sylvestris* were collected from rural areas in the region of Sfax located in the south of Tunisia. A voucher specimen (MSE 0733) was identified by a pharmacist botanist Mr. Mohamed Dammak at the Faculty of Sciences of Sfax, Tunisia.

Preparation of the *Malva sylvestris* decoction

Fresh Malva sylvestris leaves and flowers were dried and then powder-grinded. One gram of the obtained dry powder was boiled for ten minutes in one litre of water and then filtered.

Animal's treatment

Two-month-old and about 160 g body weight Wistar male rats (80 rats) were received ad libitum distilled water and standard diet (a mixture of wheat, alfalfa, soybean, vitamins, and minerals) (SICO, Sfax, Tunisia). 20 g/animal/day were used in the present study. The animals were housed in cages and maintained in a controlled environment (23°C and stable humidity) in an animal house with a constant 12 h light and 10 h darkness cycle. Control animals (C) were kept on tap water. A group (V) was given a solution of ammonium metavanadate as a sole beverage (1.185 mmol/l corresponding to 60 mg ammonium metavanadate/kg body weight). Another group (M) was given the *Malva sylvestris* decoction resulting in an intake of 0.2 g of dry plant/kg body weight/day [35], while a group (MV) was given the Malva sylvestris decoction

(similarly to group M) containing 1.185 mmol/l of ammonium metavanadate. Several methods have been reported to determine the corresponding dose of *Malva sylvestris* which can be used for humans [36], from which the dose of *Malva sylvestris* used in our experimental study corresponded to 0.018 g/kg b.w./day for humans.

All treatments were monitored during 15, 30, 60 and 90 days [37]. On day 90, animals were sacrificed and testis was fixed into Bouin's fixative, embedded into paraffin, cut into 5-µm sections and stained with hematoxyline-eosine for light microscopy.

Body and organ weights

Body weight of experimental animals was recorded on the first day before treatment (initial) and the day of sacrifice (final). Testis were dissected out, trimmed of the attached tissues and weighted. The relative weight of this organ was expressed per 100g body weight.

Chemical analyses

Serum testosterone was determined by radioimmuno-analysis using commercial kits from Immunotech (Ref, 1119). The concentration of testosterone was expressed as ng of hormone/ml. Protein concentration was estimated by the methods of Lowry et al. [38] using Bovine Serum Albumin (BSA) as the standard.

Measurement of testis lipid peroxidation

Lipid Peroxidation (LPO) was measured by the method of Yagi [39]. The level of LPO in the testicular homogenates was measured based on the formation Of Thiobarbituric Acid-Reactive Substances (TBARS). Testis sample was cut into small pieces and homogenized in 2 ml of ice-cold Tris Buffered Saline (TBS) with pH 7.4, centrifuged at 9000 xg (4°C, 20 min). Supernatants were collected and stored at 80°C until use for biochemical analyses. 125 µl of supernatants were mixed with 50 µl of TBS and 125 µl of 20% Trichloroacetic Acid (TCA) containing 1% Butylated Hydroxytoluene (BHT) to precipitate proteins and centrifuged at 1000 xg (4°C, 10 min). 200µl of the new supernatants were then mixed with 40 µl of HCl (0.6 M) and 160 µl of 120 mM Thiobarbituric Acid (TBA) dissolved in 26 mM tris-hydroxymethyl aminomethane (Tris) and the mixture was heated at 80°C during 10 min. The absorbance of the resulting supernatants was measured at 530 nm using a spectrophotometer SmartSpecTm3000 (Bio-Rad; Hercules, CA, USA).

Antioxidant Enzymes Assay

SOD activity

The method described by Asada et al. [40] was used for the assay of SOD activity. This activity was evaluated by the photo-reduction of Nitro-Blue Tetrazolium (NBT). In this assay, one unit of SOD is defined as the amount required inhibiting the photo-reduction of NBT by 50%. Riboflavin (0.26 mM) was added to start the reaction and absorbance was recorded at 560 nm using a spectrophotometer SmartSpecTm3000 (Bio-Rad; Hercules, CA, USA). The activity was expressed as unit/ mg of proteins.

Catalase activity

CAT activity was measured by the method described by Aebi [41]. The used reaction mixture (1 ml) contained 100 mM of phosphate buffer (pH 7.4), 50 mM of H_2O_2 and testis homogenate. The reaction started by adding H_2O_2 and its decomposition was observed by following the decrease in absorbance at 240 nm for one mn using a spectrophotometer SmartSpecTm3000 (Bio-Rad; Hercules, CA, USA).

GPx activity

The GPx activity was determined according to the method of Paglia and Valentine [42]. Testis homogenate was mixed

with 400 µl of 0.1 mM of glutathione (GSH) and 200 µl of 67 mM of $KNaHPO_4$ (pH=7.8). After five minutes of pre-incubation at 25°C, 200 µl of 1.3 M of H_2O_2 was added. After ten minutes, the mixture was treated with 1ml of 1% TCA and centrifuged at 3000 xg and 4°C during 10 mn. Supernatants were homogenized with 0.32 M of Na_2HPO_4 and 1mM of DTNB (5,5′-Dithiobis(2-nitrobenzoic acid). The enzyme activity was spectrophotometrically measured at 412 nm using a spectrophotometer SmartSpecTm3000 (Bio-Rad; Hercules, CA, USA) and expressed as µmoles of GSH reduced min/mg protein.

Characterization of the *Malva sylvestris* decoction

The free radical scavenger activity of the *Malva sylvestris* decoction was determined by the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) assay, as specified by Koleva et al. [43]. 1 ml of 60 µM DPPH in ethanol was added to 1 ml of different concentrations of the decoction. After thirty minutes of incubation at room temperature, the absorbance was read at 517 nm. The inhibition of Nitroblue Tetrazolium (NBT) reduction by photochemically generated O^{-2} [44] was used to determine the superoxide anion scavenging activity. The reaction mixture contained 100 µl of *Malva sylvestris* decoction added to 800 µl of phosphate buffer (pH 7.4) containing 100 µl of 96 µM NBT, 100 µl of 6.5 mM Ethylene-Diamine-Tetra-Acetic Acid (EDTA) and 50 µl of 4 µM riboflavin. After ten-minute-exposure to light, the absorbance was read at 560 nm. IC50 value is defined as the concentration (g of dry *Malva sylvestris* leaves and flowers/l) which decreases absorbance by 50%.

Quantification of Total Polyphenols

The amount of total phenolics in Malva sylvestris decoction was determined with the Folin-Ciocalteu reagent [45]. After incubation for ninety minutes at 23°C in the dark, the absorbance was read at 760 nm. Total phenolic content was expressed in mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW) using a calibration curve with gallic acid (0-400 µg mL^{-1}).

Statistical Analysis

The data was reported using the statistical package program Stat Graphics plus 5.1 (stats graphics). The results expressed are average data values plus or minus Standard Deviation (SD). The groups were compared using one-way Analysis Of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (FLSD) test. In all cases, differences were considered significant if $^{*}p<0.05$, $^{**}p<0.01$, or $^{***}p<0.001$.

Results and Discussion

Body and testis weight

The aim of this study was to examine the protective effect of *Malva sylvestris* decoction against the adverse effect of ammonium metavanadate on the reproduction of male rats and to evaluate its in vitro antioxidant potency.

TABLES 1 and 2 demonstrate the effect of ammonium metavanadate exposure on body and testis weight of male rats. In our study, the net body weight gain of vanadium-treated rats was remarkably less compared to controls. Weight loss was associated with diarrheal in 45% of treated rats and a mortality rate of 26% since the first 15 days. Other studies reported that oral administration of vanadium (0.46g/l) retarded rats' growth compared to controls. Weight loss was associated with diarrheal in 25% of treated rats and a mortality rate of 20% since the first week of treatment [10]. Previous studies

reported that there were a dose and time-dependent decrease in body weight gain in animals treated with ammonium meta-vanadate [1].

TABLE 1. **Protective effect of *Malva sylvestris* decoction on body weight during 15, 30, 60 and 90 days**.

Parameters and treatments	**C**	**V**	**M**	**MV**
15 j	177.27 ± 3.28	164.75 ± 1.35**	180.73 ± 3.62	150.72 ± 1.08**
30j	209.49 ± 3,199	187.33 ± 2,73**	207.43 ± 2,74	150.73 ± 3.74**
60j	244.66 ± 3,668	227.93 ± 3,41*	232.13 ± 3,70	198.44 ± 3.66**
90j	269.61 ± 3,343	247.83 ± 2,09*	261.01 ± 3,52	219.616 ± 2.08**

C: Control rats; **V:** Treated rat with vanadium; **(M):** Treated rat with *Malva sylvestris*; **(MV):** Rat treated with *Malva sylvestris* and intoxicated with vanadium

Values are expressed as means ± SD for eights rats in each group

One-Way Analysis Of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) as a post hoc test for comparison between groups: Comparison between (V) versus C group: $^{*}p<0.05$;$^{**}p<0.01$; $^{***}p<0.001$. Comparison between MV group versus V group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$

However, the weight gain of vanadium-treated rats given *Malva sylvestris* decoction was significantly lower than controls. These results are in keeping with those obtained by Eghosa et al. [46] who indicated that weight gain of rats treated with *Malvaceae* species was significantly lower than controls in all the pregnancy period. Reduction in the body weight of rats given *Hibiscus sabdariffa* has also been reported by Asagba et al. [47]. It seems that flavonoids from *Malva sylvestris* are not responsible for the growth effect. Dietary flavonoids did not have effects on body weight gain or food intake [48].

TABLE 2. **Protective role of *Malva sylvestris* decoction on testis weight during 15, 30, 60 and 90 days.**

Parameters and treatments	C		V		M		MV	
	AW	RW	AW	RW	AW	RW	AW	WR
15 days	1.18 ± 0.04	0.56 ± 0.02	1.09 ± 0.04	0.66 ± 0.07	1.13 ± 0.02	0.58 ± 0,02	0.96 ± 0.02	0.80 ± 0.04
30 days	1.15 ± 0.04	0.53 ± 0.02	0.86 ± 0.05	0.52 ± 0.06	1.12 ± 0.06	0.52± 0,02	1.07 ± 0.02	0.61 ± 0.03
60 days	1.26 ± 0.05	0.48 ± 0.02	1.13 ± 0.01	0.52 ± 0.02	1.29 ± 0.06	0.50 ± 0.02	1.09 ± 0.03	0.51 ± 0.02
90 days	1.16 ± 0.02	0.45 ± 0.02	1.18 ± 0.04	0.51 ± 0.02	1.21 ± 0.01	0.46 ± 0.02	1.14 ± 0.03	0.51 ± 0.03

C: Control rats; V: Treated rat with vanadium; (M): Treated rat with Malva sylvestris; (MV): Rat treated with Malva sylvestris and intoxicated with vanadium

AW: Absolute weight (g). RW: Relative weight (g/100 g body weight)

Values are expressed as means ± SD for eights rats in each group

One-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) as a post hoc test for comparison between groups: Comparison between (V) versus C group: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.Comparison between MV group versus V group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$

In the same vein, vanadium administration could not alter testis weight compared to controls. Many authors reported that single administration of 5, 15 or 25 mg/kg bw of orthovanadate did not significantly alter testis weights [49]. However, testis did not vary in vanadium-treated rats receiving *Malva sylvestris* decoction compared to controls. This organ has already attained development and its main function is producing and maturating spermatozoa as well as synthesizing

hormone. These findings confirm those obtained by Omonkhua et al. [50] who reported that *Hibiscus sabdariffa* alone had no effect on testis weight.

Lipid peroxidation

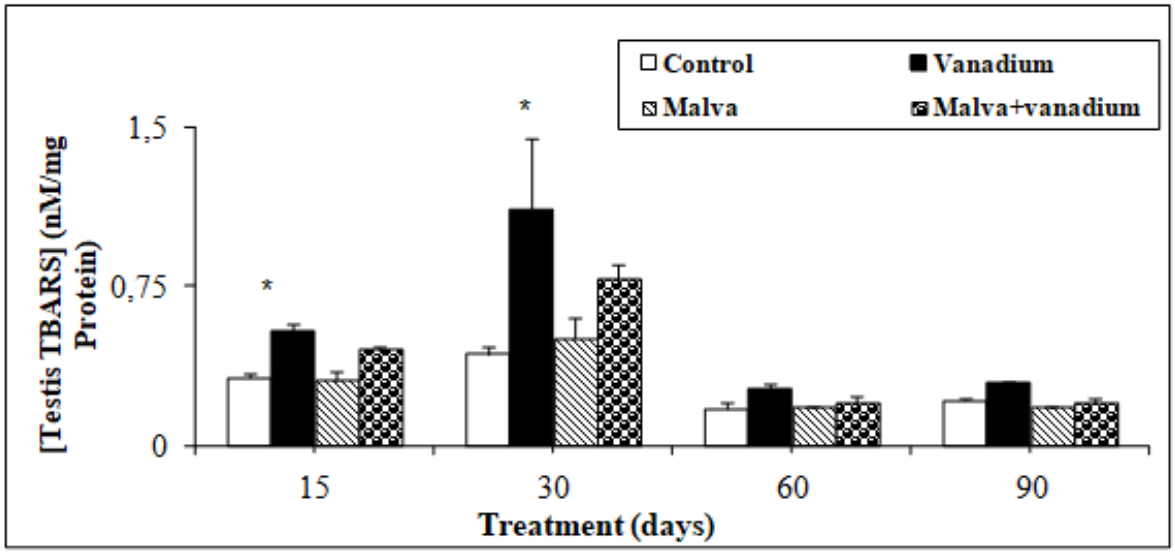

FIG. 1. **Lipid peroxidation levels (TBARS) in testis of controls and treated rats during 15, 30, 60 and 90 days.**
C: Control rats; **V:** Treated rat with vanadium; **(M):** Treated rat with *Malva sylvestris*; **(MV):** Rat treated with *Malva sylvestris* and intoxicated with vanadium
Values are expressed as means ± SD for eights rats in each group
One-Way Analysis Of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) as a post hoc test for comparison between groups: Comparison between (V) versus C group: $^{*}p<0.05$;$^{**}p<0.01$; $^{***}p<0.001$
Comparison between MV group versus V group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$

FIG. 1 reveals the level of testicular lipid peroxidation in rats subjected to different treatments. Under our experimental conditions, testicular LPO level significantly increased in 15 and 30 days after vanadium administration compared to controls. An induction of LPO by vanadium has been also noticed by other authors. Hence, oxidative damage might further contribute to the reorganization of actins, the disruption of intercellular junctions, and the destabilization of cell shape [51-53]. Chandra et al. [1] reported that vanadium treatment to rats at doses of 0.4 and 0.6 mg/kg body weight daily for 26 days caused a marked increase in the testicular LPO. Previous studies showed that vanadium increases testicular lipid peroxidation and reduces glutathione concentrations [7]. Oral administration of *Malva sylvestris* decoction prevents this vanadium induced increase of LPO level. This result suggests that phenolic compounds from *Malva sylvestris* have a significant role in the prevention of LPO. Flavonoids, widely distributed in plants, have a significant impact on tissue Reactive Oxygen Species (ROS) and were shown to have restorative effects on various oxidative stress conditions. Flavonoids stabilize testicular membranes by inhibiting the lipid peroxidation process [54].

Antioxidant enzyme activities

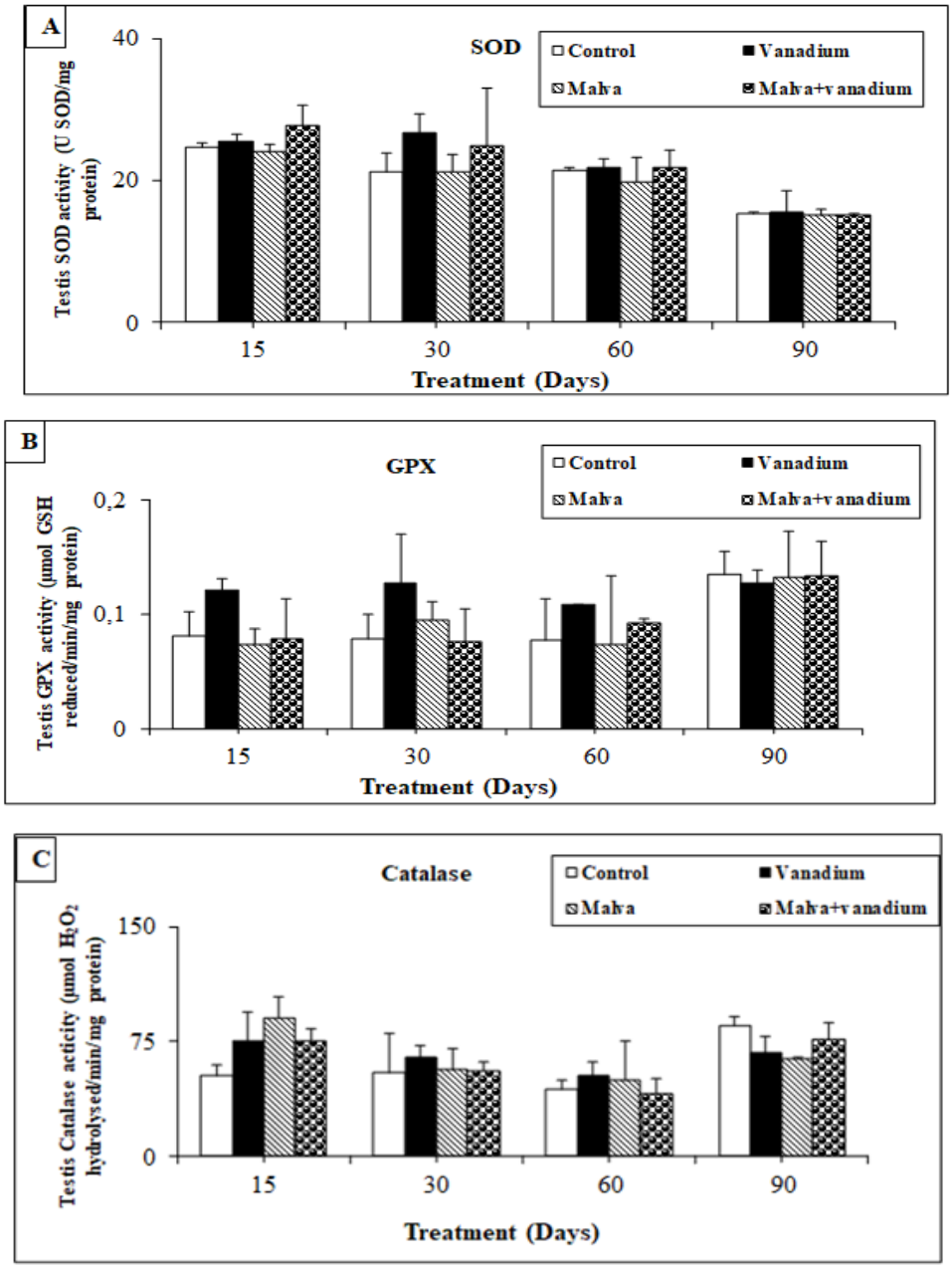

FIG. 2. **Activities of some enzymes in controls and treated rats after 15, 30, 60 and 90 days**.

(A) Superoxide Dismutase (SOD), **(B)** Catalase (CAT) and **(C)** Glutathione Peroxidase (GPX). **C:** Control rats; **V:** Treated rat with vanadium; **(M):** Treated rat with *Malva sylvestris*; **(MV):** Rat treated with *Malva sylvestris* and intoxicated with vanadium

Values are expressed as means ± SD for eights rats in each group

One-Way Analysis Of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) as a post hoc test for comparison between groups: Comparison between (V) versus C group: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. Comparison between MV group versus V group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$.

Activities of some major enzymes involved in the defense against oxidative stress, SOD, CAT, and GPX, were measured in controlling and treating rats. As shown in **FIG. 2,** no significant change was observed in testicular SOD, CAT, and GPX in different experimental groups compared to controls. The biological effects of ROS are controlled by a wide spectrum of enzymatic and non-enzymatic defense mechanisms such as SOD which catalyzes the dismutation of

superoxide anions into hydrogen peroxide. CAT detoxifies H_2O_2 and GPX which converts hydro-peroxides into nontoxic alcohols [55]. It has been reported that CAT, SOD, and GSH-Px constitute a mutually supportive team of defence against ROS [56]. Vanadium poisoning is reported to alter the activity of several enzymes involved in the defence against oxidative stress [57]. In the present study, no significant change was observed in testicular SOD, CAT and GPx in different experimental groups compared to controls. Available literature presents abundant data about the effect of inorganic and organic vanadium compounds administered to rats on the activity of some antioxidant enzymes in testis [58,59]. The changes in oxidant defense systems associated with vanadium exposure may be attributed to ineffective scavenging of H_2O_2 and thus increasing the steady-state level of oxidants in testis leading to increased lipid peroxidation [1]. Flavonoids have many functions like anti-oxidating phenol, scavenging of free radicals, chelating agents, and modifying various enzymatic and biological reactions [60]. Many of the biological actions of flavonoids have been attributed to their powerful antioxidant properties. They can act in several ways, including direct quenching of ROS, chelating of metal ions and regenerating of membrane-bound antioxidants [61]. Previous studies have shown that antioxidant activity may be dependent on the degree of hydroxylation [19]. Most of the beneficial effects of the administration of the *Malva sylvestris* decoction are due to the presence of flavonoids, which are phenolic compounds. The interference of flavonoids in the oxidation process is threefold: reacting with free radicals, chelating catalytic metals and acting as oxygen scavengers [62-64].

Serum testosterone level

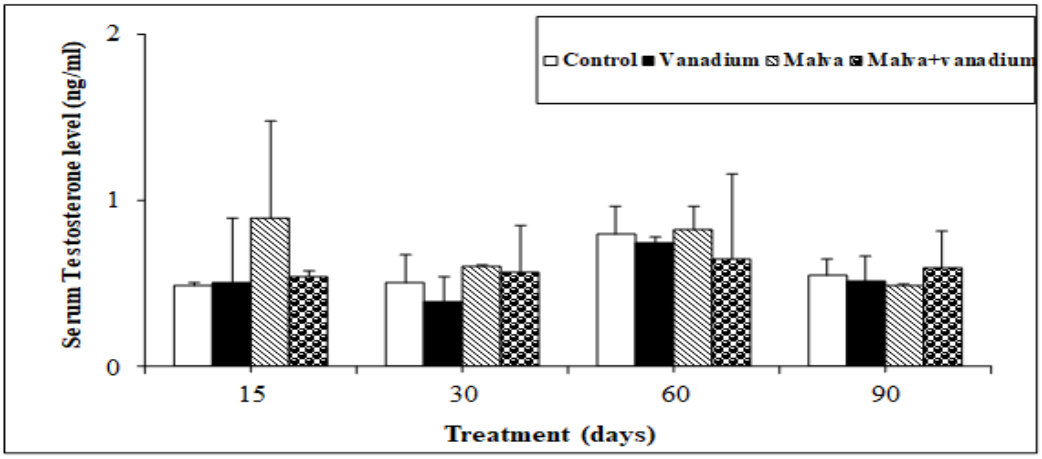

FIG. 3. **The testosterone level in serum of control and treated rats during 15, 30, 60 and 90 days**.

C: Control rats; **V:** Treated rat with vanadium; **(M):** Treated rat with *Malva sylvestris*; **(MV):** Rat treated with *Malva sylvestris* and intoxicated with vanadium

Values are expressed as means ± SD for eights rats in each group

One-Way Analysis Of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) as a post hoc test for comparison between groups: Comparison between (V) versus C group: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$. Comparison between MV group versus V group: $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$

The testosterone level in plasma of differently treated rats is shown in **FIG. 3**. Our results showed no significant difference concerning the serum testosterone level compared to the different experimental groups. Shrivastava et al. [10] demonstrated that vanadium administration induced impairment of most somniferous tubules followed by the ceasing of the spermatogenesis phenomenon. Other authors reported that vanadium tetraoxide administration for 60 days induced

ultra-structural damage, but it did not modify testosterone concentrations [65]. Similarly, our results showed no significant change in the serum testosterone level as compared to controls. This indicates that vanadium affects the exocrine function and not the endocrine. Treatment with vanadium pent-oxide did not modify testosterone concentrations secreted by a Leydig cell culture that had been LH stimulated. Such a result indicates that Leydig cells are not a target for vanadium [66]. However, *Malva sylvestris* decoction appears to prevent the abnormal features observed.

Histological finding

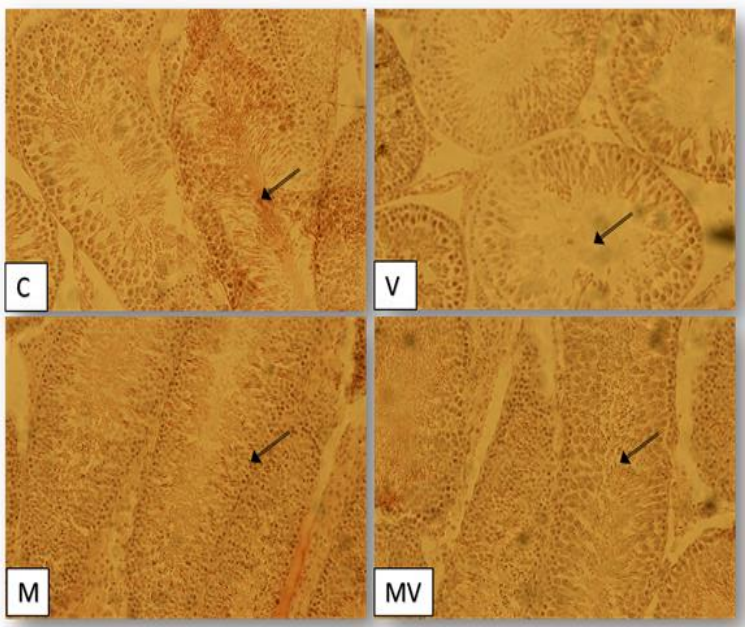

FIG. 4. **Micrographs of testis sections from controls and treated rats during 30 days.**

C: Control rats; **V**: Treated rat with vanadium; **(M):** Treated rat with *Malva sylvestris*; **(MV):** Rat treated with *Malva sylvestris* and intoxicated with vanadium

⟹ Arrows indicate the absence of spermatozoa

Spermatogenesis is a sophisticated and complex differentiation process. Degeneration of spermatogenesis is an integral and important part of normal spermatogenesis. However, spermatogonial degeneration can result from exposure to toxic chemicals [56]. In our present study, histopathological assessment results are given in **FIG. 4.** The toxic effects of vanadium on testicular histology were established. Histological examination showed normal morphological feature in the testis of controls as well as *Malva sylvestris* decoction treated groups. However, in vanadium-treated groups, some histopathologic changes were observed including atrophy of somniferous tubules and defects of spermatogenesis. Spermatozoa disappeared. These findings are confirmed by Chandra et al. [1] who reported that testicular histo-architecture of the vanadium-treated animals showed marked damages characterized by the presence of degenerating cells and germinal epithelium disruption in the somniferous tubules. As discussed above, these histopathological observations could be due to the accumulation of free radicals and the generation of their reactive metabolites, including ROS in the testis of vanadium-treated rats [67]. The administration of *Malva sylvestirs* reducing the histological alterations provoked by vanadium was quite noticeable.

***In-vitro* study**

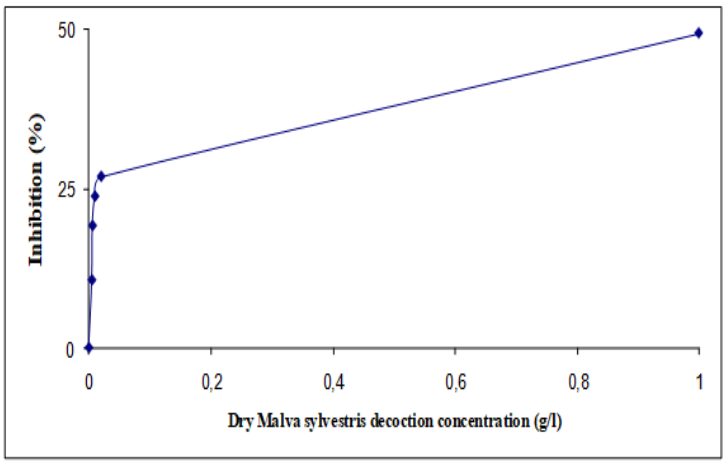

FIG. 5. **Rate of inhibition of superoxide anion radicals by *Malva sylvestris* decoction**.

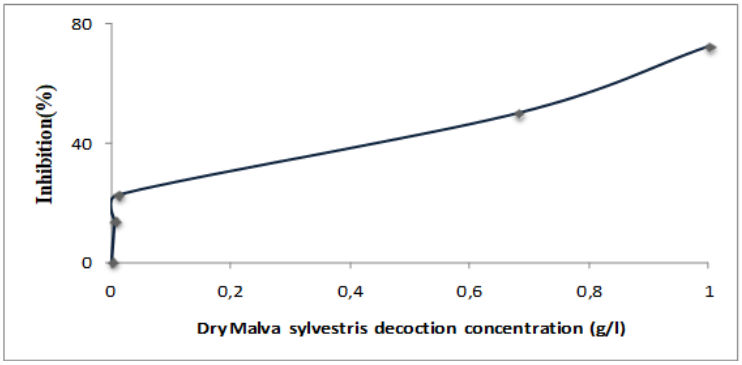

FIG. 6. **Free radical scavenger effect of *Malva sylvestris* decoction.**

Among the various methods used to evaluate the total antioxidant activity of natural compounds, the DPPH radical scavenging and the NBT reduction assays are the most commonly used. Both methods were used in the present study. *Malva sylvestris* decoction showed a notable DPPH radical scavenging activity (IC50=0.68 g of dry *Malva sylvestris*/l) which is attributed to its hydrogen donating ability (**FIG. 5**). 24% of DPPH scavenging activity was reported at 20 mg/l of aerial parts of *Malva sylvestris* [68]. Other studies investigating mallows from Turkey revealed scavenging effects on hydrogen peroxide of 46.19% at 5 g/l [69]. Annan and Houghton's [70] quantitative DPPH test on Malvaceae species indicated that Gossypium arboretum revealed antioxidant properties, with IC50 of 35.7 mg/l.

The NBT reduction assay is also positive. Malva sylvestris decoction was able to inhibit superoxide anion radical with an IC50=1 g of dry Malva sylvestris/l (**FIG. 6**). These findings suggest that flavonoids from Malva sylvestris are responsible for the antioxidant effects. Flavonoids widely distributed in plants have the ability to scavenge superoxide and hydroxyl radicals by single-electron transfer [71]. In contrast, the addition of *Malva* to the alimentation of vanadium treated rats caused a significant reduction in kidney injury, renal tissues becoming barely distinguishable from those of the healthy control rats. These results are supported by a previous study in our laboratory, where supplementation of Malva had efficient protective effects on rats [28]. This could be due to its high content of fatty acids and various sterols, particularly essential fatty acids such as omega-3 and omega-6 [72], chemical elements [73], enzymes like sulfite oxidase and catalase [74] and amino acids [75].

Phytochemical study of *Malva sylvestris* demonstrated that phenolic compounds are abundant in the plant studied. The extract contained 2.2 mg g^{-1} DW of total phenols. This important content can be related to the high solubility of phenols in polar solvents [75].

Conclusion

Data on the effects of sub-acute vanadium exposure for a long period (90 days) on oxidative stress in animals' testis are rare. Overall, our results showed that oxidative stress induced by vanadium causes testis structural and functional injuries. The consequences were tissue biochemical alteration, antioxidant enzyme activity, and oxidative stress. Our experiments proved that the co-treatment with *Malva sylvestris* decoction prevents vanadium-induced tissue damages in parallel with direct antioxidant effects. However, further study on human subjects is now needed to confirm its potential.

Acknowledgment

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Conflict of Interest Statement

The authors report no conflicts of interest associated with this manuscript.

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